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Michael D. Smith

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By Michael D. Smith

Approved:

Frederick J. Kozub
Chairman

[illegible]

Edward H. Miller

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ABSTRACT

Eight adult male albino rats were randomly assigned to each treatment condition of progesterone injections (0,10,40, and 100 mg/kg/day dissolved in 8cc of sesame seed oil), and all Ss received a hypertonic NaCl stomach loading. A control group for procedural effects was given injections of sesame seed oil alone and isotonic NaCl stomach loads. The dependent variables were body weight, food intake and water intake. Single factor analyses of covariance revealed a significant difference between the 0 mg progesterone group and the control group for food and water intake but not weight. No significant differences were found in food and water intake and weight among the four treatment conditions of progesterone. Progesterone does not enhance the efficiency of the male kidney to excrete excess serum solutes but appears to increase the osmotic stressor effect of stomach loading.

A mammal sustains life processes by regulating the intake and output of water. Water enters its internal environment through the ingestion of food and fluid and loses water to the external environment through the respiratory passages, the skin, and the elimination of wastes. Water balance is maintained in the body by multiple regulatory mechanisms that differ between and among mammalian species. One possible difference in body fluid regulation is the influence of sex hormones. This study investigates the effect of progesterone, a female sex hormone, on the response of the adult male rat to an imbalance of body fluids imposed by stomach loading hypertonic saline. At this point, however, a brief digression which summarizes the physiological evidence relevant to body fluid balance will clarify subsequent behavioral interpretations.

The body fluids are distributed into two spaces or compartments, extracellular and intracellular. According to this model (Larimer, 1968), the intracellular fluid compartment (ICF) comprises 75% of the total body fluid and the extracellular fluid compartment (ECF) comprises 25% of the total body fluid. Intracellular fluid is contained within cell membranes. Extracellular fluid is located outside cell membranes and is divided into the interstitial fluid and the blood plasma. Although these compartments are continually exchanging their chemical

contents, their respective compositions remain relatively constant over time. The chemical contents of these fluids are dependent upon the permeabilities of the capillary walls and cell membranes as well as the activities of the cellular "pumps" and cellular utilization and excretion of chemical substances.

In spite of its relatively small volume (7% of total body fluid), the plasma assumes a major role in fluid exchange and regulation. It rapidly circulates through capillary beds which provide a highly permeable and expansive surface for the exchange of fluids and constituents with the interstitial compartment. The interstitial compartment is a highly dispersed pool of fluids in which the cells of the body are bathed. The interstitial space is the center of exchange for nutrients which move between the cell and blood plasma and therefore the fluids within the cell remain the most remote from blood circulation.

The tonicity and volume of the extracellular fluid indicate the overall constancy of fluid balance within the organism. When an imbalance occurs in either of these ECF parameters, the organism's regulatory mechanisms bring the levels back within normal biological limits. Subsequent alteration of intake and/or output is made in response to these deviations of ECF tonicity and volume. Here, the output mechanism, the kidney, is considered first.

This organ is controlled by the hypothalamic-pituitary-adrenal-kidney axis. Receptor mechanisms, both neural and hormonal inform the kidney of changes in volume, then renal feedback mechanisms compensate for the responses the kidney makes to the original change. Changes in ECF pressure, distention, or arterial and venous blood flow are stimuli which indicate altered blood volume. Several specific receptor sites which affect urine output that are sensitive to these stimuli have been suggested. Gauer, Henry and Sieker (1961) found that an increase or decrease in blood volume at the left atrium of the heart caused respective increases or decreases in the amount of urine output. Davis (1961) found that a decrease in blood flow to the juxtaglomerular apparatus of afferent arterioles in the kidney caused a decrease in urine output and a conservation of sodium and water at the renal tubules. Bartter and Gann (1960) discovered that distention or constriction at the junction of the thyroid and carotid arteries produced increases or decreases in urine output.

The volume of ECF is controlled primarily by the conservation or excretion of sodium, the dominant extracellular eletrolyte. Aldosterone, a steroid hormone formed in the adrenal cortex, causes sodium reabsorption at the kidney tubules. As sodium is reabsorbed it carries water with it back into the vascular system. The mechanism for this system operates in the following manner. As ECF volume

decreases, a hormone, renin, is secreted by the juxtaglomerular cells located near the distal tubules of the kidney (Pitts, 1968; Turner, 1966). The liberated renin induces angiotension formation in the blood stream and angiotension enhances the secretion of aldosterone from the zona glomerulosa of the adrenal cortex. The increase of aldosterone causes an increase in sodium reabsorption at the distal tubule (Larimer, 1968) and obligates water reabsorption with the effect that ECF volume is restored to normal.

Other mechanisms for the regulation of volume are hemodynamic alterations such as constriction of the arterioles which causes blood to be shunted away from the capillaries. This serves to raise the blood pressure, increase the filtration rate, and provide more blood from the peripheral tissues to the core arteries and veins. Another means for regulation of volume is a change in the rate and distribution of filtration among nephrons which either increases or decreases the amount of filtered blood per unit time. Consequently, a decreasing number of operative nephrons or a slowing rate of filtration produce a diminution of filtered blood which in turn makes more fluid available to the blood stream (Pitts, 1968).

Aldosterone secretion and therefore the control of blood volume is also influenced by antidiuretic hormone (ADH). A decrease in blood volume causes the liberation of ADH from the hypothalamic hypophysial tract (Pitts, 1968). This hormone passes into the adenohypophysis via the portal

system where it promotes the production of adrenocorticotrophic hormone (ACTH), which in turn stimulates the release of more aldosterone from the adrenal gland (Sawyer et al., 1960). Moreover, increased levels of ADH enhance the effect of sodium transport at the renal tubules and induce greater aldosterone secretion at the adrenal cortex (Hilton, 1960). Thus volume changes are under multiple control, that is they are corrected by both central and peripheral mechanisms. These systems involve ADH and ACTH systems from the brain and the renin-angiotension-aldosterone system elicited at the kidney.

The tonicity of ECF is controlled principally by the ADH system. Receptor cells acting like osmometers are located in the supraoptic and paraventricular nuclei of the hypothalamus (Woodbury, 1965; Verney, 1954; Blass, 1968). Increased concentrations of ECF cause ADH to be discharged from the posterior pituitary. ADH promotes the conservation of water and elimination of solutes at the kidney tubule. Therefore, hypertonic urine is excreted and blood plasma concentrations are brought back to normal. Decreased concentrations of ECF on the other hand, cause a diminution in the discharge of ADH from the posterior pituitary so water is excreted by the kidney. This state of greater water excretion and greater reabsorption of solutes in the distal tubules and the collecting ducts serves to restore ECF concentrations back to normal (Pitts, 1968).

The mammalian organism uses adjustments in intake with the aforementioned output systems when stressed by ECF imbalance. Drinking is an important factor for adjustments in intake regulation of both volume and osmotic concentrations of the body fluids. A rat will drink when the tonicity of ECF is increased or when the volume of ECF is decreased. One method for studying the effects of internal fluid balance on the behavior of an organism is to administer hypertonic saline loads.

The administration of stomach loads of NaCl at hypertonic concentrations increases the volume and tonicity of ECF. Adult rats when subjected to this stress increase their intake of water (Gilman, 1937; Novin, 1962; Stricker, 1966). Another way the organism meets this stress is to excrete hypertonic urine and conserve water (Aldoph et al., 1954; Falk, 1955). A third adjustment available to the organism is to decrease food intake. The eating of dry food increases the tonicity of ECF (Hatton and Almli, 1969; Novin, 1962) and rats decrease their food intake with increasing concentrations of NaCl infusion (Schwartzbaum and Ward, 1958). Jacobs (1964) demonstrated that rats decrease food intake in response to 3% NaCl stomach loads and Gutman and Krause (1969) reported a significant reduction in food intake in their rats which were given 4.5% NaCl subcutaneous injections. Kozub (1972) demonstrated that adult male and ovariectomized female rats decreased their

food intake and increased their water intake 24 hours after hypertonic NaCl stomach loads. These behavioral adjustments to ECF tonicity and volume increases are in accord with the physiological mechanisms that the rat uses to establish fluid balance and they facilitate rather than retard the excretory mechanisms.

An unexpected result, however, was found with intact female rats when subjected to hypertonic stomach loads (Kozub, 1972). Intact female rats increased water intake but did not decrease food intake nor lose weight as did the male and ovariectomized female rats under these conditions of hypervolemia and hypertonicity. The results suggest that differences exist in the regulatory mechanisms of male and female rats. Of the many possible explanations for the differences in behavior of male and female rats to dipsogenic stimuli the difference in circulating sex hormones is an obvious first choice. The following discussion provides a possible mechanism in which the concentration and action of sex hormones present a mediational system through which the regulation of body fluids can differ.

As discussed earlier, hypertonic NaCl increases the tonicity of ECF and activates the ADH system thereby inducing the elimination of excess solutes and conservation of water. When ECF solute levels are very high however, the excretion of excess solutes may cause a net water loss

(Blass, 1968). Consequently, a condition of hypovolemia results which initiates ECF volume mechanisms for regulation; namely, the renin-angiotensin-aldosterone system. Aldosterone, it will be recalled, promotes the reabsorption of sodium ions with obligatory water uptake so this system seemingly works antagonistically to correct the imbalance of hypertonicity and hypervolemia. Antidiuretic hormone (ADH) also stimulates the secretion of ACTH which in turn serves to increase the output of aldosterone. Furthermore, aldosterone secretion is increased by the action of circulating ADH on the adrenal glands. It is apparent then, that while ADH restores ECF balance it also antagonizes its own effect by stimulating the reabsorption of sodium at the kidney tubule through the aldosterone system. But, the antagonistic effect and the effect of renal hypovolemia subserves osmotic restoration because hypertonic urine is excreted from the kidney due to the influence of ADH (Stricker, 1969).

A chemical agent which blocks the sodium-retaining action of aldosterone can enhance the ability of an organism to restore osmotic balance rapidly. If the kidney operates with maximum efficiency; for example, without the interference of aldosterone, then the additional burden of food consumption on the osmotic properties of ECF should be diminished.

Progesterone may be the mediating chemical agent which interacts with aldosterone. Progesterone has been shown to inhibit the action of aldosterone by a process of competitive inhibition at the renal tubule of the kidney (Sharp and Leaf, 1966; Uete and Venning, 1963). In agreement with this action aldosterone excretion increases upon the administration of progesterone (Landau et al., 1955, 1958, 1961; Laidlaw et al., 1962). Progesterone also impedes the manufacture of aldosterone (McKerns and Bell, 1960; Müller, 1971). Furthermore, the rise in secretion and excretion of aldosterone during pregnancy is largely a consequence of increased progesterone secretion (Venning et al., 1957; Jones et al., 1959).

The male sex hormone, testosterone, is ineffective as an inhibitor of aldosterone at its production site (McKerns and Bell, 1960) or at the level of the kidney (Thorn and Engel, 1938). There was no difference between estrogen and progesterone and progesterone alone on the effect of aldosterone excretion (Laidlaw et al., 1962). Hence progesterone seems to be the major inhibiting agent of aldosterone and therefore provides the intact female rat with the capacity to rapidly excrete excess ECF solutes. Male rats and ovariectomized female rats due to insufficient amounts of progesterone must reduce food intake in order to compensate for this decreased efficiency of excreting hypertonic urine..

In agreement with the hypothesis about aldosterone inhibition, Kozub (1972) provided results which estimated and supported the predicted differences in level of kidney function. He used the equation which Corbit (1969) proposed to describe the drinking of the rat in response to hypertonic NaCl administration.

$$D = k\left[\frac{n}{\alpha} - v\right] \quad \text{where}$$

D is the amount drunk,

k is the contribution of the kidney to osmotic regulation and is a function of the time after loading,

n is the number of millimoles of effective osmotic solute,

v is the amount of water in milliliters in which the solute is dissolved,

α is the effective osmotic solute dissolved in the body fluids of a S in water balance. For the rat $\alpha = 0.15M$.

Assuming that the rat is in osmotic balance when the measure of D is taken and given that the values of n and v are known from the experimental procedure, the value of k can be computed. A k of 1.0 means the rat drank enough to restore osmotic balance and the kidney made no appreciable contribution for eliminating excess solutes. A k of 0.0 means the kidney eliminated all the excess solutes and drinking did not appreciably affect body fluid balance. If k equals 0.25, the kidney eliminated 75% of the excess solute in the time the S drank 25% of the water required for isotonicity.

The results of the Kozub study showed the female kidney excreted 86% of the load in the time it took the male kidney to excrete 75% of the load and the ovariectomized female rat's kidney did not perform significantly different from the male's. It is readily apparent then, that not only did the female intake responses differ from the male intake responses but the kidney of the female rat was more efficient in eliminating excess solutes than the kidney of the male rat. This evidence supports the idea that a part or all of the differences observed in male and female rat intake responses are a function of kidney efficiency, and the present hypothesis asserts that progesterone is the mediator in this functional relationship between intake and output.

To complicate the issue, however, alternative effects of progesterone have been identified which deserve consideration and suggest other explanations for the difference in male - female responsiveness to dipsogenic stimuli. Müller (1971) suggests that progesterone is a bio-chemical precursor in the manufacture of aldosterone; consequently, increased concentrations of progesterone may enhance the production of aldosterone (Laidlaw et al., 1962). Also, the structural similarities of these compounds might account for the relatively weak salt-retaining capacity of progesterone. Such an action has been demonstrated in dogs (Thorn and Engel, 1938) and suggested in rodents in which progesterone in large amounts supports the life of the adrenalectomized animals

(Gaunt et al., 1938). The maintenance of weight and food consumption manifested by female rats then, is achieved in spite of antagonistic effects of progesterone. The influence of progesterone on aldosterone may not be a simple inhibitory relationship as proposed earlier but may be an interaction relationship of inhibition then facilitation depending on levels of hormone circulating in the blood plasma.

The facilitative influence of progesterone at the kidney actually might be an artifact of neuroendocrine systems which mediate energy balance in an animal. Changes in energy balance would be revealed in altered food intake, weight and body weight composition. Hervey and Hervey (1967, 1966, and 1964) have demonstrated that upon administration of progesterone in normal and ovariectomized female rats, weight gain and increased food consumption were observed. There is considerable evidence that active levels of estrogen cause the opposite effect. Estrogen has been shown to excite the ventral-medial hypothalamus (VMH) which in turn inhibits the lateral hypothalamus (LH) attributed to be the excitatory center for thirst and hunger (Myer and Thomas, 1967; Wade and Zucker, 1970). Estrogen then seems to inhibit the hunger and thirst centers in the brain of the female rat so under the influence of active levels of this hormone the organism decreases the amount it eats and drinks. In agreement with this effect, Tarttelin and Gorski (1971) showed

body weight and food and water intake decreased during estrus and increased during metestrus and diestrus in the adult female rat. These response fluctuations relate directly to the cycle of estrogen secretion from the ovary. When active levels of progesterone are injected into the female rats, food consumption and weight increases. Progesterone appears under this condition to interact antagonistically with the restraining influence of estrogen on food intake and weight (Wade and Zucker, 1969). Rodier (1971) further demonstrated not only an inhibitory influence of progesterone on food intake and weight gain but also showed that progesterone retards running activity of the female rat in the activity wheel during estrus. Increased running activity during estrus parallels in a temporal fashion the period of increased circulating estrogen. Myer and Thomas (1967) asserted that progesterone may eliminate the effect of estrogen on the VMH by a process of competitive inhibition which allows longer periods of excitation in the LH eating center. While these effects of progesterone on the female rats energy balance are sufficiently clear, it produces little or no change in weight and food intake in the castrate and intact male rat (Hervey and Hervey, 1964, 1966), except for a small gain of water in the tissues of the body.

The purpose of this study is to establish the influence of progesterone on the response of the adult male rat to hypertonic stomach loading. If progesterone is the

principal agent which enhances the efficiency of the female kidney for eliminating hypertonic urine and it does not affect the energy balance in male rats, then the administration of this hormone should augment the response of the male kidney to excrete excess ECF solute and not affect energy balance. Male rats when injected with progesterone after hypertonic stomach loads should continue eating and manifest only slight weight changes in comparison to their baseline on these measures and in comparison to subjects which do not receive progesterone.

METHOD

Subjects. Forty male albino rats were obtained from a commercial supplier and tested at approximately 90 days of age.

The Ss were maintained throughout the course of the experiment under conditions of constant light, ad libitum food (Purina powdered chow) available from standard cup dispensors and water available from standard laboratory water bottles or from stoppered polypropylene graduated cylinders as the experimental conditions required. The range in temperature of the laboratory was 75 ± 3°F. Each S was used only once and during the course of the experiment was housed individually.

Design. Four levels of progesterone injection (0,10,40, 100 mg/kg/day) comprised the treatment levels. Eight Ss were randomly assigned to each treatment condition and all Ss received a hypertonic NaCl stomach load immediately after their respective progesterone injections. A fifth group of eight Ss also randomly assigned received injections of 0 mg of progesterone and istonic NaCl stomach loads as a control for procedural effects. The dependent variables were body weight, food intake and water intake.

The control group also provided a measure for the variables other than those directly manipulated in this experiment which have been shown to affect weight and

water and food intake. For example, Budgell (1970) demonstrated that water intake increased as a function of ambient temperature, so even routine fluctuations in daily temperature may influence the rats consumption of water. Kakolewski and Deaux (1970, 1971) demonstrated that stress induced by handling or rotation in a drum caused body fluid osmolality to increase in rats to the degree that drinking increased and eating was disrupted. Since all rats in this study were handled and stressed during weighing, anethetizing and injecting procedures, it was important to run a control group which provides an estimate of these effects. The method of anethetizing each animal with ether before the injections and stomach loadings has been shown to influence drinking in the rat. Czech, Wayner and Gawronski (1969) showed that ether depresses the salt arousal of drinking in rats. Although their experimental conditions measured the effect of ether administered after stomach loading which was not identical to the present design (ether administered before load), the effects of ether anesthesia necessitated the addition of the control group. Another variable shown to affect the intake responses of rats are the diurnal influences of the light dark cycle verses the constant light cycle. Hardy (1970) found that a constant light cycle caused persistent estrus in female rats which suggests an alteration of endogenous sex hormone concentrations. Sex hormones,

it will be recalled, have been shown to influence eating. The constant light cycle might conceivably have an effect on the hormone levels of male rats. Although this question has not been studied directly, Oatley (1967) demonstrated that the well documented diurnal variation of water and food intake observed in rats can be explained on the basis of a variation in food intake alone. Hence, the light and dark or constant light cycles can affect intake behavior in response to stomach loads sufficiently to warrant the inclusion of a control group. Finally, the influence of intestinal verses stomach distention factors was shown by Balagura and Coscina (1969) to significantly affect the meal eating patterns of rats. Kakolewski and Deaux (1970) provided evidence that even sham stomach loading caused a sufficient increase in serum osmolality to initiate drinking. Therefore, these and a number of undetermined variables all contribute to the rats response in this design and make the addition of a control group essential for a more accurate evaluation of the experimental conditions.

Procedure. The dependent variables of body weight, food intake and water intake were recorded for 24 hour periods. There were two 24 hour periods: the baseline session and the experimental session. Recordings were taken between 10:00 PM and 12:00 AM during three consecutive evenings

and were made to the nearest 1 gm or 1 cc. After the recordings were taken at the beginning of the baseline session, each S was anethetized with ether then given the injection of progesterone dissolved in sesame seed oil or the solvent alone depending on the treatment classification of S. The progesterone injections were administered at dosages of 0,10,40,100 mg/kg/day. Each injection was prepared so that the amount of solution equaled .8 cc. The solution was injected subcutaneously at two sites in the back region to prevent excessive irritation. The control and 0 mg progesterone groups received .8 cc of sesame seed oil. Immediately after the injections the animals were placed back in their home cages until the beginning of the experimental session.

The procedures used during the baseline session were duplicated for the start of the experimental session except the stomach loads were administered to the rats just prior to placement back into the home cage. Each S in a progesterone treatment group was given a hypertonic stomach load of 3% b.w. and 10% NaCl (wt. X vol.). Each S in the control condition was given an isotonic stomach load of 3% b.w. and .9% NaCl (wt. X vol.). The solutions were prepared with distilled water and were loaded through an orally inserted catheter (rubber tube .07 in. outside diameter) connected to a syringe. At the end of the experimental session, recordings were taken.

Treatment of Data. A single factor analysis of covariance was run on the three types of data; weight, water and food intake. The first analysis for each type of measurement was on the four progesterone groups to determine the effect of progesterone injections on the dependent variables. The second analysis for each type of measurement examined the differences between the isotonic and the hypertonic 0 mg progesterone groups. This analysis provided information about the effects of procedure on the dependent variables as described in the design.

The baseline measure for each animal served as the covariate and the measure for the experimental session served as the variate in the analysis of covariance. This method was chosen to provide statistical control for the variability due to experimental error which otherwise could not be controlled in the present design. Sources of constant error such as differences in animal size are removed by this method and increase the precision of the experiment.

The trend effects of stomach loading on the three dependent variables are depicted in graphic form as mean percent changes from baseline. These values were computed by taking the difference of the experimental and the baseline measurements divided by the baseline and multiplying the result by 100 for each animal. The average

of these percent changes for each group represents the mean values plotted in each figure.

RESULTS

A single factor analysis of covariance was run on the three dependent variables for the four progesterone injection groups and an analysis of covariance was run on the three dependent variables for the 0 mg progesterone groups which received hypertonic and istonic stomach loads. The mean percent changes from baseline for all the groups on the three dependent variables were graphed showing the trend effects of the two independent variables, NaCl stomach loads and dosages of progesterone injection.

The results in Table 1 show no significant difference was found in the analysis of covariance for water intake among the four progesterone treatments. In Table 2 a significant difference ($p < .01$) was found between the hypertonic and istonic stomach load conditions. The 0 mg progesterone group which received the hypertonic stomach load drank significantly more water than the isotonic 0 mg progesterone group as can be seen in Figure 1. Furthermore, the remaining progesterone groups receiving hypertonic stomach loads also differed from the isotonic control condition by the same magnitude: approximately a 65% increase in water intake. The control condition showed virtually no change from baseline. The slight differences in water intake between the four progesterone conditions can be seen with the 40 mg group showing the smallest increase in water intake at 59% above baseline.

Table 2

ANALYSIS OF COVARIANCE ON WATER INTAKE (CC) FOR THE ISOTONIC AND HYPERTONIC NACL STOMACH LOAD GROUPS EACH RECEIVING 0 MG OF PROGESTERONE

Source of Variation	df	MS	F
Between Treatments	1	4407.0	125.9*
Within Treatments	13	34.9	
Total	14		

*$p < .01$

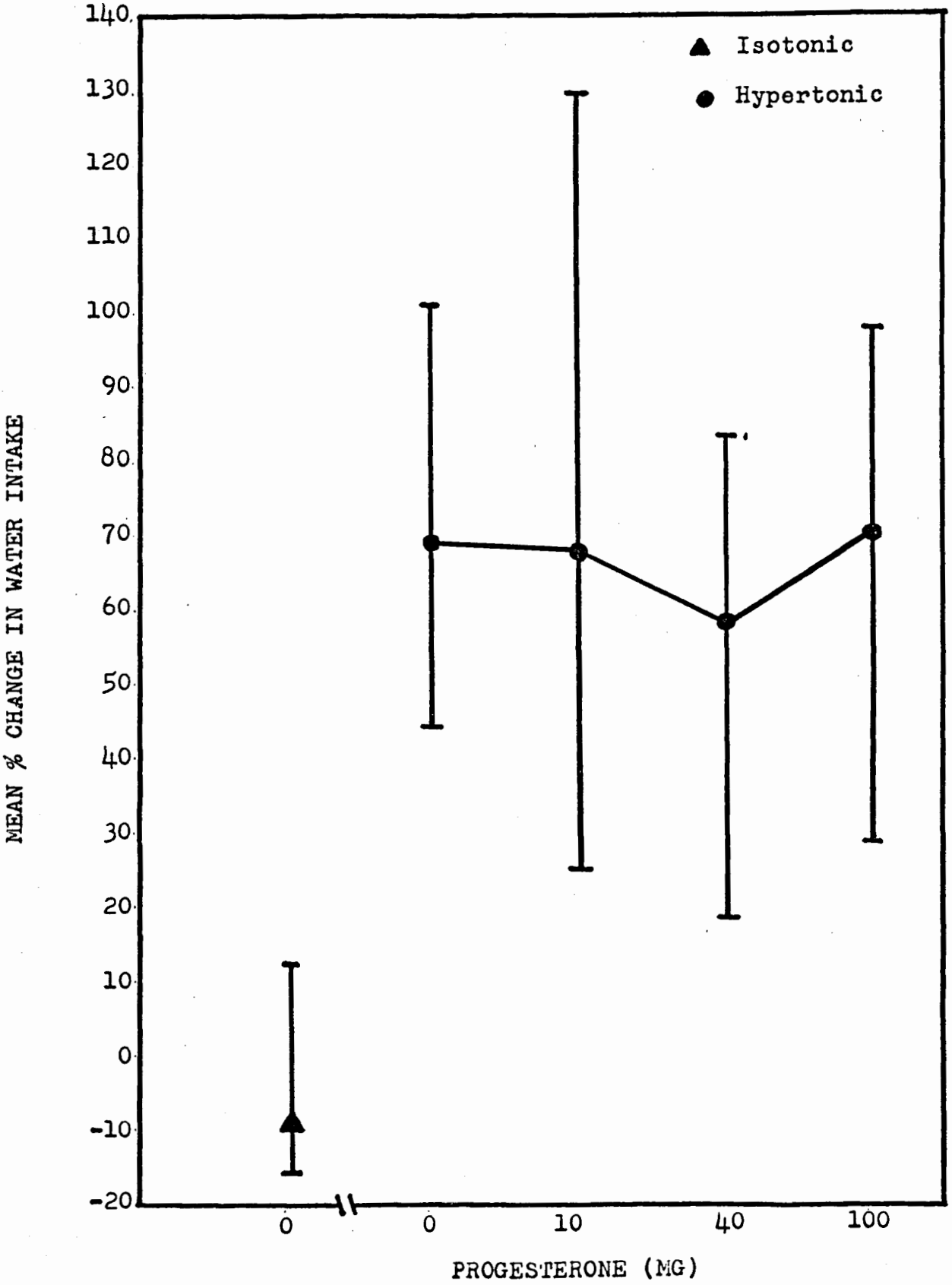

Figure 1. Mean % change in water intake as a function of progesterone and NaCl isotonic and hypertonic stomach loads for 24 hrs. (vertical lines represent ranges)

Table 3 shows no significant difference for food intake among the four progesterone treatments contrary to the expected results for the present hypothesis under investigation. According to the hypothesis, the groups receiving progesterone injections should eat more food than the 0 mg group because progesterone would enhance the ability of the kidney to excrete excess blood serum solutes. Therefore, it would permit these groups to show no decrement in food intake compared to the 0 mg progesterone group because the osmotic burden of the hypertonic stomach load would be removed quicker, and increase the animal's tolerance for the additional osmotic stress that food would cause. The expected difference among groups was not found. However, even though the analysis indicates there is no significant difference, the mean per cent changes in food intake from baseline seen in figure 2 show that progesterone injections caused more of a reduction in food intake than seen in the 0 mg group, with the greatest difference found between 0 mg and 10 mg of -11%. These results suggest that not only did progesterone not enhance the ability of the kidney to eliminate excess solutes but the hormone had the effect of forcing the male rats to further reduce food intake as a method to reach internal balance. Thus, progesterone injections seem to cause an osmotic stressor effect on food intake.

Table 3

ANALYSIS OF COVARIANCE ON FOOD INTAKE (GM) FOR THE PROGESTERONE INJECTION GROUPS EACH RECEIVING HYPERTONIC NACL STOMACH LOADS

Source of Variation	df	MS	F
Between Treatments	3	2.2	.07
Within Treatments	27	31.8	
Total	30		

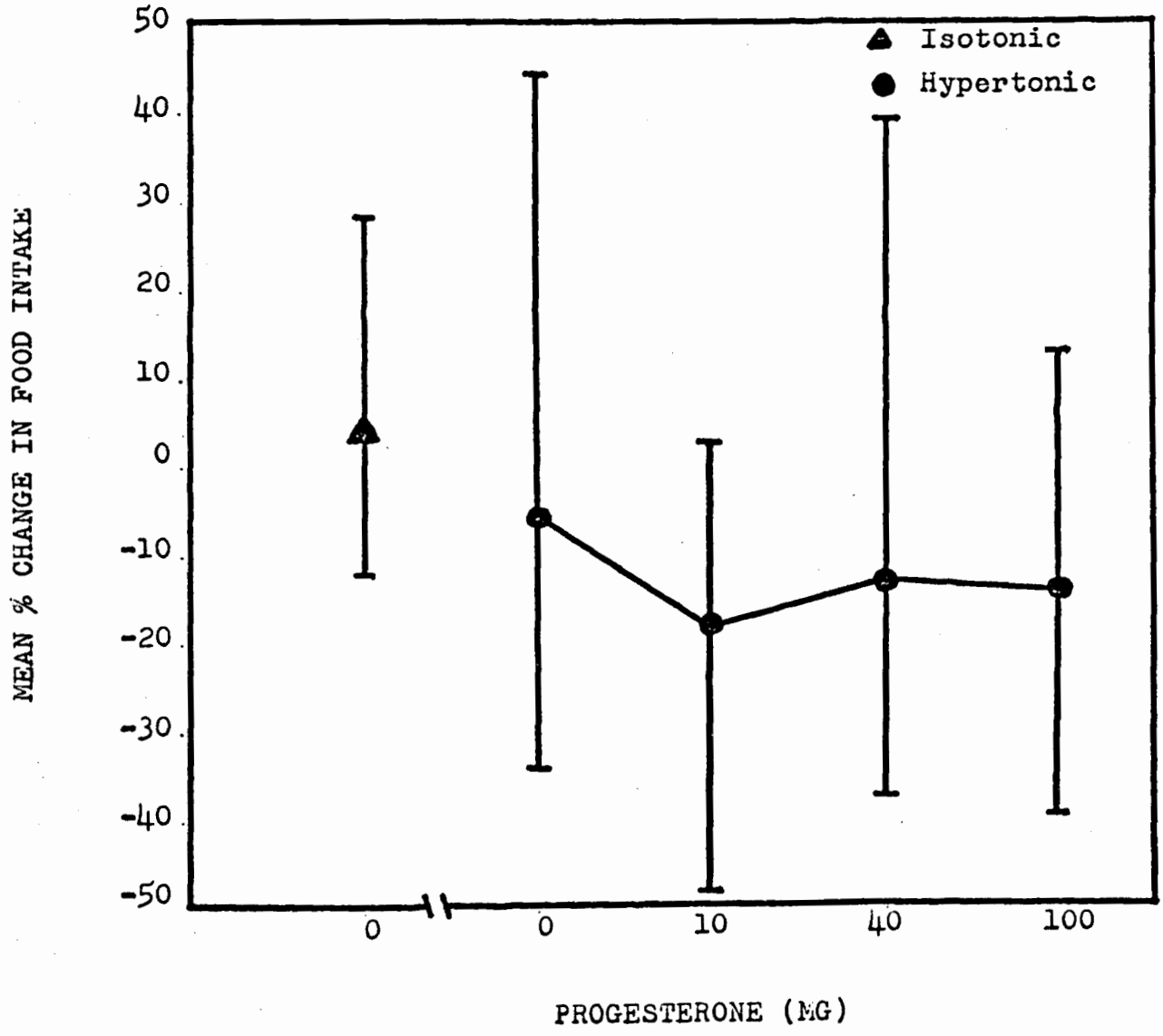

Figure 2. Mean % change in food intake as a function of progesterone and NaCl isotonic and hypertonic stomach loads for 24 hours (vertical lines represent ranges)

The results in Table 4 for the analysis of covariance between the hypertonic and the isotonic stomach load treatments indicate a significant difference in food intake. Although the margin of -8.5% change in food intake from baseline for the hypertonic stomach load group is not as large as that shown in previous studies, the size of the group and the level of significance obtained clearly show a reliable effect. The absolute difference of '15% change in food intake seen in figure 2 between the isotonic and hypertonic stomach load groups illustrates that a pronounced relation between hypertonic and isotonic stomach loading is achieved despite the length of the measurement period. The measurement taken 24 hours after loading probably reduced real difference in food intake.

Table 5 shows no significant difference in weight among the four progesterone treatment conditions. Again, if the present hypothesis were to have been confirmed, the analysis of covariance would have revealed a significant difference in weight among the groups: male rats in the 0 mg condition would lose more weight than the groups which received progesterone injections. Instead, as seen in figure 3, the 0 mg group did not lose weight in comparison to the baseline but actually gained a slight amount of weight during the 24 hour period after stomach loading. The 10, 40, and 100 mg groups decreased in weight from .25% to .50%

Table 4

ANALYSIS OF COVARIANCE ON FOOD INTAKE (GM) FOR THE ISOTONIC AND HYPERTONIC NACL STOMACH LOAD GROUPS EACH RECEIVING 0 MG OF PROGESTERONE

Source of Variation	df	MS	F
Between Treatments	1	142.6	10.9*
Within Treatments	13	13.1	
Total	14		

* $p < .01$

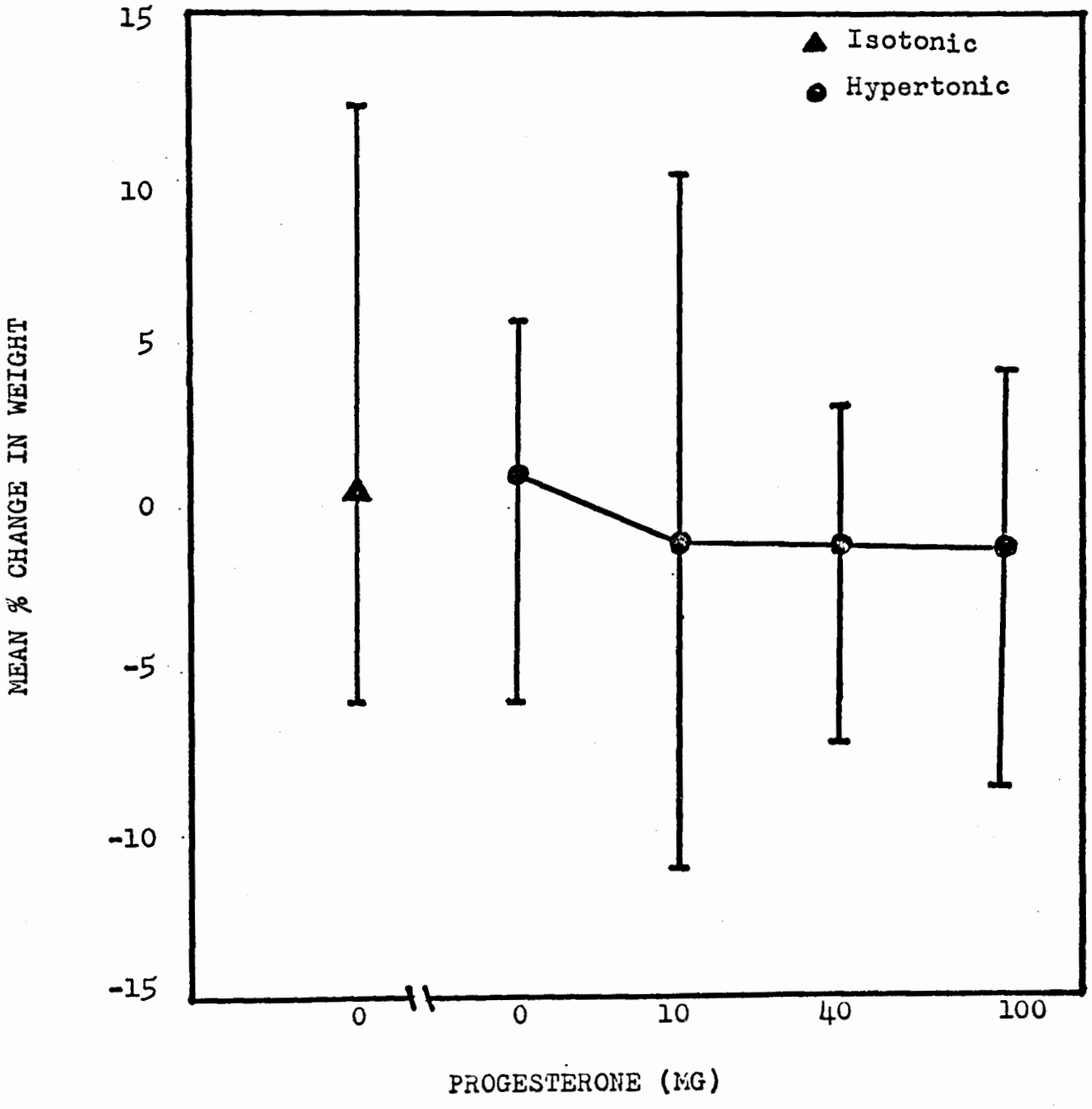

Figure 3. Mean % change in weight as a function of progesterone and NaCl isotonic and hypertonic stomach loads for 24 hours (vertical lines represent ranges)

Table 5

ANALYSIS OF COVARIANCE ON WEIGHT (GM) FOR THE PROGESTERONE INJECTION GROUPS RECEIVING HYPERTONIC NACL STOMACH LOADS

Source of Variation	df	MS	F
Between Treatments	3	19.4	.81
Within Treatments	27	23.8	
Total	30		

Table 6

ANALYSIS OF COVARIANCE ON WEIGHT (GM) FOR THE ISOTONIC AND HYPERTONIC NACL STOMACH LOAD GROUPS EACH RECEIVING 0 MG OF PROGESTERONE

Source of Variation	df	MS	F
Between Treatments	1	2.5	.10
Within Treatments	13	25.7	
Total	14		

after stomach loading. Although a significant difference was not obtained in the analysis, inspection of Figure 3 shows a similar trend in weight decrease as the trend observed for decrease in food intake. The groups receiving progesterone both decreased food intake and decreased in weight more than the 0 mg group. The similarity of these trends adds support to the notion that progesterone caused an additional osmotic burden for the male rat. The magnitude of these changes in food intake and weight for the 0 mg group do not reach the levels of previous research so conclusions about the stressor effect of progesterone in the adult male rat are not justified at this time. Furthermore, the lack of a significant difference in weight change between the isotonic and hypertonic stomach load conditions shown in Table 6 is at variance with other studies. The 0 mg group which received a hypertonic stomach load, should have lost weight in the order of -5.0% from baseline and should not have gained the .35% in weight found in this study. These results call into question the validity of the assumption that weight loss in response to hypertonic stomach loads reflects a decrease in eating. In this study the overall difference of weight change in the isotonic and hypertonic groups ranged from .35% to -.50%, well within normal day to day weight fluctuations.

Inspection of the raw data for weight and for food intake in all groups showed no significant deviations in

range or variability. Consequently, these results are not due to abnormal responses of one or several rats which confound real relationships existing between the independent and dependent variables. The underlying functions between variables implied by the results of this study are considered in the next section.

DISCUSSION

The present results show progesterone does not increase the ability of the male rat to excreet excess solutes after hypertonic stomach loading. In fact the data suggest it actually had an osmotic stressor effect although the results of this trend did not reach statistical significance. In keeping with the hypothesis that progesterone augments the ability of the rat to excrete excess serum solutes, the groups receiving progesterone should have shown less weight loss and no change in food intake compared to the 0 mg progesterone group. Instead the progesterone groups gave a greater weight loss and decrease in food intake than the 0 mg progesterone group suggesting not the predicted facilitory effect but the effect of adding a further osmotic burden.

These results differ from the findings in another study. Kozub (1972), found adult male rats increased water intake 86%, decreased food intake 48% and lost weight 5% in response to hypertonic stomach loads. Here the comparable group, 0 mg progesterone, while increasing water intake 70% and decreasing food intake 9% did not lose weight. The isotonic stomach load group which served as a control for procedures did not deviate significantly from baseline. Thus the animals in this study did not lose weight as found in previous research.

The outcome of this study seems reliable, namely, progesterone has a slight stressor effect in male rats. The failure of progesterone injections to influence elimination of excess serum solutes might be attributed to organ insensitivity. It is well documented that critical stages exist in the life of a mammal when circulating hormones organize tissues in structure and create specific sensitivities to chemical substances in various parts of the body and brain (Wade and Zucker, 1970). Research on the influence of sex hormones in neonatal and prenatal male and female rats shows that circulating levels of sex hormones can affect brain organization and preset or eliminate a sensitivity to these chemicals at a later time in the life of the animal. Progesterone injections may not have an impact on the organs or internal chemistry of the adult male rat due to this lack of sensitivity. Instead the injected progesterone may merely act as an additional osmotic stressor. Owing to the paucity of research in this area, a number of preliminary exploratative studies need to be performed before any statements about the organizational and consequent sensitivity effect of progesterone can be accepted.

Another facet of this study which needs investigation due to the scarcity of research is the establishment of a biologically active dosage level of progesterone in male

rats. It could be argued that none of the dosage levels of 10, 40, and 100 mg/kg/day of progesterone approximated the level of hormone necessary to achieve a facilitory effect in the male rat even though these dosage levels have been shown to be effective in the female rat (Hervey and Hervey, 1967; Rodier, 1971). Exploratative and descriptive research is needed to determine the functional relationship of progesterone injections verses food and water intake and weight. To the author's knowledge there are no data on the influence of progesterone in male rats which measures all three of the dependent variables used in this study. Not only would a delineation of dosage levels be an important first step but information about the time relationships for accomplishing this active level of progesterone would assist in the design of future studies.

A limitation in this study which might have altered the findings are the baseline operations; specifically, the use of one 24 hour period for determining the ad libitum food and water intake and weight measures prior to the experimental session. Perhaps a better more stable estimate of baseline could have been achieved by taking these measurements over a longer period and using an average value on each variable as more representative of where the animal should be if body functions were not interupted. Once a more reliable baseline is found the statistical

analysis would become more precise. If the animal was younger than 80-90 days, however, measurements before and after the experimental session would have to be used for baseline estimation in order to control for the natural growth function seen in the maturing rat.

The inconsistancy between the present findings and those of previous research (Kozub, 1972) call into question the relationship that is assumed to exist between food intake and weight. In the former study loss of weight was attributed to be a result of a decrease in food intake. But the failure to replicate these results in this study prompts a re-evaluation of the relationship between weight loss and food intake.

Again the lack of sufficient data in this area and the inconclusive results of both this study and the former research make a critical evaluation of the intake and weight functions impossible. A measurement taken 24 hours after stomach loading gives an overall measure on the dependent variables but does not establish the temporal relationships needed for determining how the dependent variables interrelate and vary over time with one another. A study in which measurements are taken more frequently, e.g. every two hours for 24 hours on all three dependent variables, would isolate the influence of decreasing food intake and weight.

According to the present hypothesis, the kidney of the female rat eliminates the osmotic burden of the stomach

load faster than in the male rat. If this is the case then shorter interval measurements ought to disclose the manner in which the female rat maintains ad libitum levels of food consumption after hypertonic stomach loading as shown in the Kozub (1972) study. Does the female rat continue eating food after loading at the same rate as before loading in apparent disregard for the increasing tonicity of blood serum, or does the female rat merely resume eating sooner than the male rat does and, if so, do these delayed eating behaviors of male and female rats differ in rate? These are only a few of the questions which must be answered before any statement can be accepted about the relationship between food intake and weight.

To further clarify the relationships between kidney efficiency and weight and food intake measures, the assumption that progesterone enhances the ability of the female kidney to excrete excess serum solutes must be checked by direct measurements of urine concentration. The present hypothesis holds that the male rat eats less food and loses weight because it cannot excrete a urine of as great a concentration as the female rat. While the present hypothesis about the enhancing effect of progesterone on the kidney may be true and future studies using urine analysis may show the urine of the female rat is more hypertonic than the urine of the male rat, inferences drawn on the basis of food intake and weight seem spurious. The

results of the present study showed that the group which did not receive progesterone after stomach loading did not lose a significant amount of weight nor decrease its food intake to the same extent reported in previous research. Thus, the difference in these results suggests conclusions drawn from food intake and weight about kidney efficiency need a closer examination especially with the addition of a more direct measure, urinalysis.

Other intervening variables besides food intake may influence weight. For example, water intake, movement of fluids within the body fluid compartments, and excretion both urinary and fecal could account for weight losses. In male rats a decrease in weight of 5% for a 24 hour period shown by Kozub (1972) probably reflects much more than a decrease in food intake. A part or all of the weight decrease may be caused first, by a movement of fluids from the intracellular to the extracellular spaces to reduce rising serum tonicity, then secondly, by subsequent loss of these fluids at the kidney. Following this reasoning, greater fluid loss at the kidney in male rats could be caused by some physical property which prevents a greater concentration of urine and not just a deficiency produced by a prevalence of aldosterone as argued earlier. A physical property, such as a difference in the counter-current mechanism (Pitts, 1968) would reduce the ability of the male kidney to excrete urine

of the same concentration as the female kidney, but a full discussion of this possible difference cannot be addressed here.

In the male, a greater influx of fluids from the intracellular and interstitial compartments to the blood stream would offset a rise in serum tonicity that results from a deficiency in sodium excretion. The female rat would lose less intracellular and interstitial fluids with a more efficient kidney. Under these conditions a greater loss of body fluid weight would be reflected in greater water consumption. This predicted difference in water intake, male rats drinking proportionately more water than female rats, was found in the Kozub (1972) study (males drank 36% more water than females). A large difference in water intake strongly suggests more intracellular fluid had to be replenished in the male rat. Weight loss may show that the male rat merely has not drunk enough water to restore body fluid to the baseline level in addition to the slight deficits caused by a decrease in food intake. In summary, the significance of weight loss in relation to food intake cannot be adequately anchored in the mechanisms for excretory processes to make definitive statements about kidney efficiency. Measurements on input behavior are not sufficient for determining the differences in the efficiency of the male and female kidney and the influence of progesterone on kidney function.

Future studies must include not only measurement on input behaviors and weight but urinalysis and fecal measurement as well.

This study was designed to test the hypothesis that progesterone enhances the efficiency of the adult male kidney to excrete excess serum solutes. The results suggested that progesterone does not facilitate the response of the male rat after hypertonic stomach loading, but instead, it acts as an additional stressor to the already burdened system of the male rat. This outcome, however, did not make the hypothesis about progesterone facilitation in female rats untenable. But, it did call into question some of the underlying assumptions assumed to exist between the behavioral measures of food intake and weight. It also provided grist for alternative explanations that will require future research and the use of more direct measures of kidney efficiency, such as urinalysis. Ultimately, more research will reveal that no single chemical agent or mechanism is responsible for kidney function but rather a complex interplay of systems, behavioral, structural and chemical of which progesterone is only one, all contribute to the maintenance of body fluid balance.

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VITA

Michael D. Smith, the author, was born in Minneapolis, Minnesota on February 21, 1946. He lived in Rockford, Illinois until age 16 when he moved to New Delhi, India to live with his family. Upon graduation from the American International School in 1964 he entered the Virginia Polytechnic Institute and was awarded the B.S. degree in psychology in June, 1969. In September, 1969 he entered the University of Richmond and began work toward the degree of Master of Arts in psychology. Presently he is employed as a Court Psychologist for the Chesterfield County Juvenile and Domestic Relations Court. Prior to this position he was employed for three years at the Virginia State Penitentiary as an Assistant Psychologist.